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Molecular Evidence for Nosocomial Transmission of Hepatitis C Virus in a French Hemodialysis Unit

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A systematic virological follow-up of hemodialysis patients identified 11 cases of de novo hepatitis C virus (HCV) infection in the same unit that were not due to blood transfusion. There were three groups of infection, each occurring within a period of 3 months: four infections with genotype 1b, two infections with genotype 1b, and five infections, four with genotype 1a and one with genotype 5a. The possibility of patient-to-patient transmission was addressed by sequencing the first hypervariable region of the HCV genome in sera taken shortly after infection. Phylogenetic analysis indicated clustering of most of the cases of de novo infections. Sequence homologies identified potential contaminators among already infected patients. All patients who were infected with closely related HCV isolates were found to have been treated in the same area and during the same shift or on the previous one. These infections could have been due to occasional breaches of the usual hygiene measures. Strict adhesion to hygiene standards and routines, continuously supervised, remains the key rule in the management of dialysis patients. Nevertheless, the isolation of patients with HCV could reduce the risk of infection because occasional lapses of preventive hygiene measures or unpredictable accidents can always take place in a hemodialysis unit. This policy needs to be evaluated by large-scale prospective studies. ***J. Med. Virol. 58:139–144, 1999.***
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INTRODUCTION

Infection with hepatitis C virus (HCV) remains a substantial clinical problem in hemodialysis patients despite the obvious risk reduction provided by testing blood donors for anti-HCV antibodies and the use of recombinant erythropoietin to treat anemia. Several studies indicate that there is nosocomial transmission of HCV in these patients, despite rigorous preventive measures [Irie et al., 1994; Okuda et al., 1995]. The prevalence of antibodies to HCV has been reported to increase with the time on hemodialysis [Giammaria et al., 1992; Hardy et al., 1992; Huang et al., 1993; Dussol et al., 1995] even in patients who had not been transfused. Convincing evidence for nosocomial infection also comes from several prospective studies on hemodialysis patients [Jadoul et al., 1993; Simon et al., 1994]. Lastly, patients on peritoneal dialysis and those on home hemodialysis are at less risk of HCV infection than are patients dialyzed at a center [Barril and Traver, 1995; Dussol et al., 1995].

Virological monitoring of patients treated by maintenance hemodialysis has been greatly improved by the introduction of a standardized reverse transcriptase–polymerase chain reaction (RT-PCR) assay that directly detects HCV RNA in the serum [Young et al., 1993]. This test has recently been licensed for clinical use; it distinguishes between current and past infection in anti-HCV–positive patients and shortens the "window" between infection and seroconversion. Standardized molecular techniques for HCV genotyping have also been developed [Stuyver et al., 1993]. Because the number of HCV genotypes is limited, merely identifying a viral genotype is not sufficient to show how the virus was transmitted to an individual patient. However, analysis of the sequence of variable regions of the HCV genome does identify a virus population in an infected individual and provides a powerful means of tracing the source of infection [Allander et al., 1994; Esteban et al., 1996].

A prospective study, including a systematic virological follow-up of hemodialysis patients, was initiated in January 1994 at Toulouse-Rangueil University Hospi-

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tal, France. There were several episodes of acute HCV infections that occurred in the same unit over a 12-month period. Newly infected patients and those already infected were investigated by genotyping by a reverse hybridization assay and by sequencing the first hypervariable region (HVR-1) of the HCV genome. The results provided unequivocal evidence for the nosocomial transmission of HCV.

MATERIALS AND METHODS

Patients

All the hemodialysis patients attending the center of Toulouse-Rangueil University Hospital, France, were followed prospectively using the same diagnostic tools as part of an ongoing program that began in January 1994. The program included monthly biochemical tests, with measurements of serum alanine aminotransferase (ALT) and virological studies using anti-HCV screening, as well as tests for HCV RNA every 3 months and at each episode of cytolysis.

The 70 patients included in the study were dialyzed for 4 hr, three times a week. The unit was divided into two areas: one contained seven individual rooms (area A) and the other had two rooms, each with four beds (areas B and C). In the first area there were three shifts per day, and two shifts per day in the other area. Each area had its dedicated pool of nurses, who wore gloves when working with patients. Patients were not isolated according to their HCV status. The dialysis machines were not dedicated to a given patient. Dialysis machines were chemically disinfected with Dialox (CFPO, Paris, France). The dialyzers were never reused. Heparin vials were not shared.

Serological Testing

Anti-HCV screening was performed using two third-generation enzyme immunoassays (Ortho Diagnostic Systems, Roissy, France; Sanofi Diagnostic Pasteur, Marnes-La-Coquette, France). A third-generation immunoblot RIBA-3 (Chiron, Emeryville, CA) was used as a supplementary test.

HCV RNA Detection

Serum HCV RNA was detected using the Amplicor HCV RNA qualitative assay (Roche Diagnostic Systems, Neuilly, France) according to the manufacturer's instructions. The cutoff value of the assay was 1,000 RNA copies per ml of serum.

HCV Genotyping

The Line Probe Assay (LiPA HCV II; Innogenetics, Zwijndrecht, Belgium) was used; this is a reverse hybridization assay based on variations in the highly conserved 5′ noncoding region. The six major genotypes (1–6) and their subtypes were identified and listed according to the nomenclature of Simmonds et al. [1994].

Sequencing of HCV Isolates

Two-strand direct sequencing was carried out on a nested PCR product in the E2 gene encompassing the HVR-1 region. RNA was extracted from 100 μl of serum using guanidinium thiocyanate-phenol-chloroform. Reverse transcription was carried out with 20 units of M-MuLV reverse transcriptase (Boehringer-Mannheim, Mannheim, Germany) and an antisense primary primer. PCR was carried out under standard conditions with 2.5 units of *Taq* polymerase (AmpliTaq, Perkin Elmer Cetus, Norwalk, CT). Outer primers were KS2 (antisense primer, 5′-TTGCAGTTTAAGGC-AGTCC-3′) and KS1 (sense primer, 5′-CAGGGACTG-CAATTGCTCAATCTA-3′); inner primers were KS4 (antisense primer, 5′-ATGTGCCAGCTGCCATTGGT-3′) and KS3 (sense primer 5′-CACTGGGAGTCCTG-GCGGG-3′). The cycling parameters of the primary PCR were: initial denaturation at 95°C for 7 min; 30 cycles with denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and polymerization at 72°C for 90 sec; final elongation at 72°C for 5 min. An aliquot (5 μl) from the resulting mixture was used for a 30-cycle nested PCR under the same amplification conditions. The KS3-KS4 amplification gave a 212-bp product that was purified with QIAamp columns (Qiagen, Courtaboeuf, France) and subsequently sequenced in the sense and antisense directions by the dideoxy chain termination method (ABI PRISM Ready Reaction AmpliTaq Fs, Dye Deoxy Terminators, Applied Biosystems, Paris, France) on an ABI377 automated DNA sequencer (Applied Biosystems). All contamination prevention measures suggested by Kwok and Higushi [1989] were strictly applied.

Phylogenetic Analysis

E2 sequences were first aligned with the multiple sequence editor CLUSTAL W version 1.6 [Thompson et al., 1996]. The sequences were gap-stripped and the pairwise matrix was generated with the DNADIST program in the PHYLIP version 3.572 c package [Felsenstein, 1995]. Tree topology was inferred by neighbor-joining with the Kimura two-parameter distance matrix (PHYLIP) with a transition/transversion ratio of 2.0 and drawn with TREEVIEW version 1.4 [Page, 1996]. The bootstrap analysis was performed with CLUSTAL W (1,000 resamplings) to place approximate confidence limits on individual branches. The numbers at the nodes indicate the frequency with which the node occurred in 100 bootstrap replicates.

Nucleotide Sequence Accession Numbers

Complete sequences of HVR-1 have been sent to EMBL: accession numbers Y15528–Y15566 and AJ007975–AJ007985.

RESULTS

ALT, Anti-HCV Antibodies, and HCV RNA

At the beginning of the study (January 1994), a total of 70 patients were treated by dialysis in the unit. The prevalence of anti-HCV authenticated by RIBA-3 was 26/70 (37.1%) and HCV RNA was detected in 22/26 (84.6%) of the anti-HCV–positive patients. The HCV

TABLE I. Characteristics of 11 De Novo Infections Occurring in Patients Treated in the Same Hemodialysis Unit Within a Period of 12 Months[a]

Sex	Age (years)	Room	Date of de novo infection (month, year)	ALT elevation Start (xULN)	ALT elevation Peak (xULN)	ALT elevation Acute cytolysis (months)	Anti-HCV antibodies delay after ALT elevation (months)	HCV RNA at first elevation	HCV genotype
F	33	A	May, 94	3.1	13	5	12	+	1b
F	43	A	May, 94	6.4	6.4	5	4	+	1b
M	23	A	May, 94	4.0	4.2	6	8	+	1b
M	45	A	June, 94	14.9	14.9	3	0	+	1b
M	73	C	December, 94	1.3	1.3	5	12	+	1b
F	64	C	December, 94	1.1	4.4	4	8	+	1b
M	41	A	February, 95	2.9	3.5	4	4	+	5a
M	70	B	February, 95	1.3	3.3	4	12	+	1a
M	69	B	April, 95	1.1	1.1	2	0	+	1a
M	68	A	April, 95	1.6	1.6	4	12	+	1a
F	62	B	April, 95	2	3	3	8	+	1a

[a]ALT, alanine aminotransferase; ULN, upper limit of the normal value.

genotypes were 1b (n = 14), 1a (n = 4), 2a/2c (n = 2), and 4 (n = 2). None of the anti-HCV–negative hemodialysis patients was found with serum HCV RNA.

The number of patients treated by dialysis varied during the study period as a consequence of transplantation, death, and new admissions. All the patients undergoing dialysis were routinely screened for anti-HCV antibodies and HCV RNA. A total of 11 de novo infections occurred between May 1994 and May 1995 (Table I). De novo infections occurred in both areas of our unit: six in area A and five in areas B/C. All the patients suffered from an episode of acute cytolysis that lasted on average 4 months. The first significant increase in ALT was 1.1–14 times the upper limit of the normal value (mean, 4.8), and the mean peak of ALT was 5.4 times the upper limit of the normal value. Anti-HCV antibodies appeared within 3 months of the rise in ALT. Only two patients had anti-HCV antibodies at the time of the first significant increase in ALT. By contrast, HCV RNA was always detected before anti-HCV, coinciding with the increase in ALT. The HCV genotypes in newly infected patients were 1b (n = 6), 1a (n = 4), and 5a (n = 1). The temporal relationships between de novo infections showed that four infections with genotype 1b occurred in May–June 1994; two infections with genotype 1b occurred in December 1994; the remaining five de novo infections occurred between February and April of 1995, four with genotype 1a and one with genotype 5a.

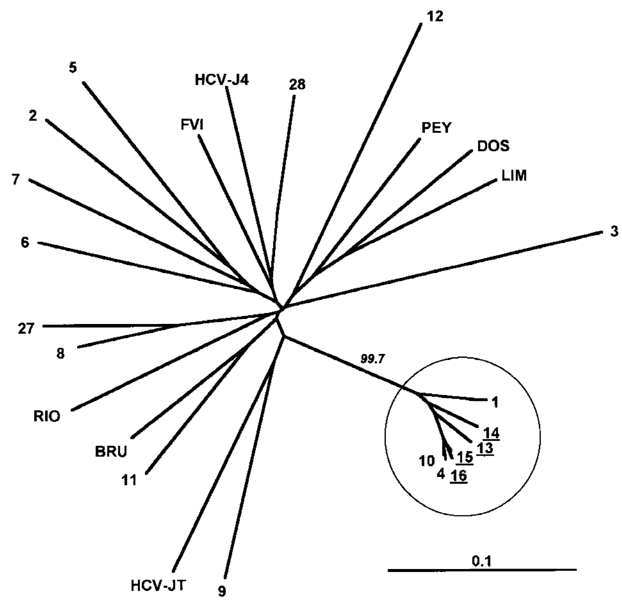

Fig. 1. Neighbor-joining phylogenetic tree analysis comparing nucleotide sequences in the E2 encompassing the first hypervariable region in HCV genotype 1b isolates from the 4 hemodialysis patients infected in May–June 1994 (patients 13, 14, 15, and 16), the 14 already infected hemodialysis patients (patients 1 to 12, 27, 28), local controls (PEY, FVI, RIO, BRU, DOS, LIM), and controls from the EMB data bank (HCV-JT accession number D01171 and HCV-J4 D10750). All sequences from the four newly infected patients and sequences from three already infected patients clustered together in a monophyletic nest. The number given at the branch point indicates the frequency with which the node occurred out of 100 bootstrap replicates.

Analysis of First Hypervariable Region of Viral RNA

Serum samples were taken from all HCV RNA-positive hemodialysis patients at the time of the first group of de novo infections (May–June 1994). HCV genotype 1b isolates from newly infected patients and those from already infected patients were analyzed by sequencing the HVR-1 region of the E2 gene. Figure 1 shows the phylogenetic tree for all 1b isolates from hemodialysis and a control panel. The latter consisted of 1b isolates from unrelated nonhemodialysis patients with hepatitis C from the same geographical area plus 1b isolates extracted from the EMBL data bank. The analysis provided strong evidence that the four isolates from recently infected patients and three other isolates from already infected dialysis patients were closely related (mean pairwise nucleotide genetic distance: 0.053; bootstrap value: 99.7%). Sera were taken from all HCV RNA-positive hemodialysis patients at the time of the second group of de novo infections with HCV genotype 1b (between December 1994 and Febru-

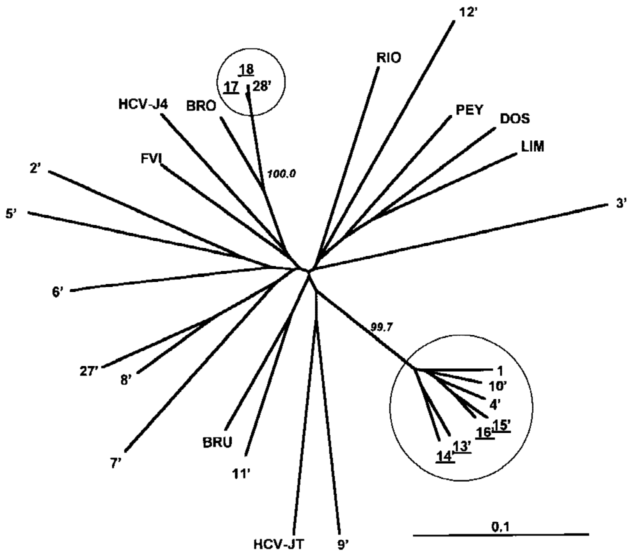

Fig. 2. Neighbor-joining phylogenetic tree analysis comparing nucleotide sequences in the E2 encompassing the first hypervariable region in HCV genotype 1b isolates from the 2 hemodialysis patients infected in December 1994 (patients 17 and 18), the 4 hemodialysis patients infected in May–June 1994 (patients 13', 14', 15', and 16'), the 14 already infected hemodialysis patients (patients 1' to 12', 27', 28'), local controls (PEY, FVI, RIO, BRU, DOS, LIM), and controls from the EMB data bank (HCV-JT accession number D01171 and HCV-J4 D10750). Sequences from the two newly infected patients in December 1994 clustered in a monophyletic nest. The numbers given at the branch points indicate the frequencies with which the node occurred out of 100 bootstrap replicates.

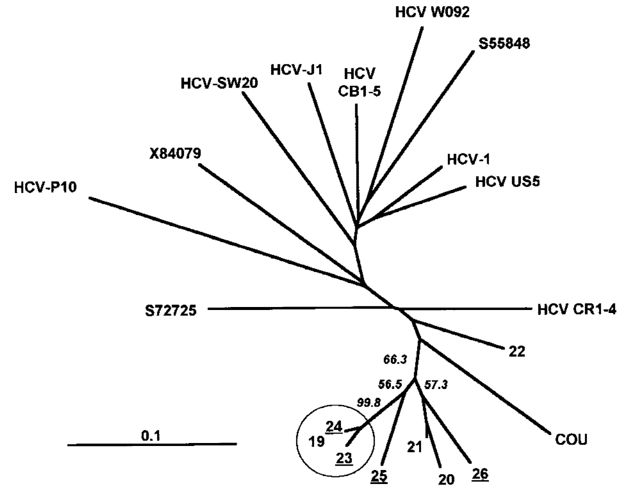

Fig. 3. Neighbor-joining phylogenetic tree analysis comparing nucleotide sequences in the E2 gene encompassing the first hypervariable region in HCV genotype 1a isolates from the four hemodialysis patients infected between February–April 1995 (patients 23, 24, 25, and 26), the four already infected hemodialysis patients (patients 19, 20, 21, 22), a local control (COU), and controls from the EMB data bank (HCV-1 accession number M62321, HCV-J1 D10749, HCV-P10 U14232, HCV-SW20 L16891, HCV-CB1-5 L19371, HCV-W092 A27609, HCV-US5 M74808, HCV-CR1-4 L19373, S72725, X84079, S55848). The numbers given at the branch points indicate the frequencies with which the node occurred out of 100 bootstrap replicates.

ary 1995). Analysis of the HVR-1 region from 1b isolates revealed two clusters (Fig. 2). The first cluster included the seven isolates from patients whose sera collected 6 months before contained virus with very similar sequences. The second cluster included three isolates, two from the newly infected patients and one from an already infected patient (mean pairwise nucleotide genetic distance: 0.009; bootstrap value: 100%). Phylogenetic analysis of the HVR-1 region from 1a isolates circulating at the time of the third group of HCV infections (February to April 1995) showed that three isolates, of which two were from newly infected patients, were closely related (mean pairwise nucleotide genetic distance: 0.015; bootstrap value: 99.8%) (Fig. 3). The two remaining type 1a isolates from newly infected patients were less similar to this cluster.

Risk Factor Analysis and Review of Procedures

None of the newly infected hemodialysis patients had had a blood transfusion within 6 months before their first HCV RNA-positive serum was collected. There was no correlation with invasive procedures such as transplantation, dentistry, or endoscopy. The nurses and medical staff were all HCV antibody-negative throughout the study. Only one patient who had acquired a de novo infection with an HCV genotype 5a, genotype not recognized among HCV-infected patients from our unit, admitted using intravenous drugs. For the first group of HCV infection, the four newly infected patients were dialyzed in the same area (area A), on the same day, and on the same shift. Two of the three potential contaminating patients were also dialyzed in the same area, on the same days, and on the same shifts. The third was dialyzed in the same area and on the same day, but on the previous shift. In the second episode, the two newly infected patients as well as the potential contaminator were dialyzed in the same area (area C), on the same day, on the same shift. And in the last episode, three newly infected patients and one potential contaminator were dialyzed in the same area (area B), on the same day, and on the same shift. The last newly infected patient and two potential contaminators were dialyzed in the same area (area A), on the same day, and on the same shift.

All the nurses and medical staff were interviewed and there had been no obvious breach of standard procedures: cleaning and disinfection of machines, instruments and surfaces; avoidance of sharing articles between patients; handwashing; systematic use of gloves. All intravenous medicines used during dialysis, including the heparin, were in single-dose units. After this dramatic outbreak of acute hepatitis C infection in our unit, we reinforced preventive hygiene measures, with special emphasis on the educational program. The result is that we have had only two de novo cases of hepatitis C infection in dialysis patients who had received no blood transfusions over the last three years, again suggesting nosocomial transmission.

DISCUSSION

Molecular evidence was found for the nosocomial transmission of HCV in a hemodialysis unit during a prospective study. This report is unique in identifying

a large number of recorded HCV de novo infections among a selected group of patients undergoing chronic hemodialysis. There was no correlation with blood transfusion or any other invasive procedure. There was no staff-to-patient HCV transmission, as recently reported in another setting [Esteban et al., 1996]. The issue of patient-to-patient infection was addressed by HCV genotyping and sequence analysis of the HVR-1 region of HCV RNA-positive hemodialysis patients together with a control panel of HCV isolates from unrelated patients with chronic hepatitis C. Molecular studies have been carried out retrospectively by others to reconstruct the route of infections within dialysis units [Sampietro et al., 1995; De Lamballerie et al., 1996; Stuyver et al., 1996]. Regions of the HCV genome with lower variability than HVR-1 were analyzed because, in contrast to our study, sera taken shortly after infection were not available.

Genotyping provided an initial HCV characterization. Only one patient was infected with HCV having a unique genotype (type 5a), which was not found in already infected patients being treated in the hemodialysis unit. As this patient was also an intravenous drug user, HCV transmission probably occurred outside the dialysis environment. Analysis of the sequence of a highly variable region of the HCV genome in samples taken from patients infected with the same genotype shortly after infection provided evidence for clustering of most of the cases of de novo infection. The isolates of each cluster had very similar sequences. The four 1b isolates from patients infected in May–June 1994 differed from each other by less than five nucleotides. These four isolates were also closely related to three isolates from already infected patients. All these patients were dialyzed on the same area and on the same shift, or on the one prior to that of a potential contaminator. The same cluster was identified in samples taken 7–11 months later, excluding the possibility of laboratory contamination. Examination of the paired sequences from the same patient showed little variation (0–8 nucleotide substitutions; data not shown). In the second cluster, two 1b isolates from the newly infected patients and one 1b isolate from an already infected patient differed by only two nucleotides. These three patients were all dialyzed in the same room, which was different from that in which the former 1b patients were treated, and on the same shift. The HCV sequences in the third cluster involving 1a isolates showed that they were closely related to patients who were dialyzed in the same area and on the same shift.

We believe these infections occurred because of occasional breaches of universal precautions, as described in other settings [Allander et al., 1995]. Contamination of surfaces and instruments with small amounts of HCV-infected blood together with insufficient infection control procedures, particularly during busy periods or in the case of an emergency with a patient, could have led to HCV transmission. No new HCV infections were recorded in the unit until 1997, probably because of increased awareness. However, two new infections occurred in the second semester of 1997; this led us to implement the universal hygiene routines by the introduction of separate rooms and machines for HCV-infected patients. Our study raises concern about the spread of HCV in dialysis units, despite the adoption of the usual hygiene measures. The isolation of HCV-positive patients on hemodialysis is controversial [Calabrese et al., 1991; Jadoul et al., 1993; Jadoul, 1995]. Strict adherence to hygiene standards and routines, continuously supervised, remains the key rule in the management of dialysis patients. Nevertheless, the isolation of patients with HCV could reduce the risk of infection because occasional lapses of preventive hygiene measures or unpredictable accidents can always take place in a hemodialysis unit. Eradicating HCV from dialysis patients awaiting transplant by alpha-interferon treatment could also reduce the risk of the nosocomial spread of the virus [Izopet et al., 1997].

In conclusion, phylogenetic analysis of the nucleotide sequences in a hypervariable region of the HCV genome from newly infected hemodialysis patients and those already infected on the same unit provided strong evidence for patient-to-patient transmission. This study shows that nosocomial transmission of HCV can occur even in developed countries where equipment for hemodialysis such as needles, tubes, and dialyzers are disposable and the staff is usually well educated and aware of the risk of transmission of blood-borne viruses. Continuing education programs, written procedures for hygiene routines, including sterilization procedures, and the involvement of nursing and medical staff in the careful surveillance of hygiene routines are the key to the control of HCV transmission in dialysis environments. Whether or not the use of dedicated areas and dialysis monitors for HCV-positive patients helps prevent HCV transmission to non–HCV-infected patients, particularly in centers with a high prevalence of HCV infection, needs to be evaluated by large-scale prospective studies.

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REFERENCES

Allander T, Medin C, Jacobson SH, Grillner L, Persson MAA. 1994. Hepatitis C transmission in a haemodialysis unit: molecular evidence for spread of virus among patients no sharing equipment. J Med Virol 43:415–419.

Allander T, Gruber A, Naghavi M, Beyene A, Soderstrom T, Bjorkholm M, Grillner L, Persson MAA. 1995. Frequent patient-to-patient transmission of hepatitis C virus in a haematology ward. Lancet 345:603–607.

Barril G, Traver JA. 1995. Prevalence of hepatitis C virus in dialysis patients in Spain. Nephrol Dial Transplant 10(suppl 6):78–80.

Calabrese G, Vagelli G, Guaschino R, Gonella M. 1991. Transmission

of anti-HCV within the household of haemodialysis patients. Lancet 338:1466.

De Lamballerie X, Olmer M, Bouchouareb D, Zandotti C, De Micco P. 1996. Nosocomial transmission of hepatitis C virus in haemodialysis patients. J Med Virol 49:296–302.

Dussol B, Perthezene P, Brunet P, Berland Y. 1995. Hepatitis C virus infection among chronic dialysis patients in the south of France: a collaborative study. Am J Kidney Dis 25:399–404.

Esteban JI, Gomez J, Martell M, Cabot B, Quer J, Camps J, Gonzalez A, Otero T, Moya A, Esteban R, Guardia J. 1996. Transmission of hepatitis C virus by a cardiac surgeon. N Engl J Med 334:555–560.

Felsenstein J. 1995. PHYLIP (Phylogeny Interference Package) Version 3.56. Seattle: Department of Genetics, University of Washington.

Giammaria U, De Meo F, Acitelli S, Tancredi M, Daniele M, Barnabei R, Maschio C. 1992. HCV infection in hemodialysed patients: incidence and correlation with dialysis age. Nephron 61:335–336.

Hardy NM, Sandroni S, Danielson S, Wilson DJ. 1992. Antibody to hepatitis C virus increases with time on hemodialysis. Clin Nephrol 38:44–48.

Huang CS, Ho MS, Yang CS, Lee CL, Tan CA. 1993. Hepatitis-C markers in hemodialysis patients. J Clin Microbiol 31:1764–1769.

Irie Y, Hayashi H, Yokozeki K, Kashima T, Okuda K. 1994. Hepatitis C infection unrelated to blood transfusion in hemodialysis patients. J Hepatol 20:557–559.

Izopet J, Rostaing L, Moussion F, Alric L, Dubois M, T That H, Payen JL, Duffaut M, Durand D, Suc JM, Puel J. 1997. High rate of hepatitis C virus clearance in hemodialysis patients after interferon-α therapy. J Inf Dis 176:1614–1617.

Jadoul M. 1995. Hepatitis C virus. Lancet. 345:189–190.

Jadoul M, Cornu C, van Ypersele De Strihou C, Group UC. 1993. Incidence and risk factors for hepatitis C seroconversion in hemodialysis: a prospective study. Kidney Int 44:1322–1326.

Kwok S, Higushi R. 1989. Avoiding false positives with PCR. Nature 339:237–238.

Okuda K, Hayashi H, Kobayashi S, Irie Y. 1995. Mode of hepatitis C infection not associated with blood transfusion among chronic hemodialysis patients. J Hepatol 23:28–31.

Page RDM. 1996. TreeView version 1.4. Glasgow: Division of Environmental and Evolutionary Biology, University of Glasgow.

Sampietro M, Badalamenti S, Salvadori S, Corbetta N, Graziani G, Como G, Fiorelli G, Ponticelli C. 1995. High prevalence of a rare hepatitis C virus in patients treated in the same hemodialysis unit: evidence for nosocomial transmission of HCV. Kidney Int 47:911–917.

Simmonds P, Alberti A, Alter HJ. 1994. A proposed system for the nomenclature of viral hepatitis C viral genotypes. Hepatology 19: 1321–1324.

Simon N, Courouce AM, Lemarrec N, Trepo C, Ducamp S. 1994. A twelve year natural history of hepatitis C virus infection in hemodialyzed patients. Kidney Int 46:504–511.

Stuyver L, Rossau R, Wyseur A, Duhamel M, Vanderborght V, Van Heuverswyn, Maertens G. 1993. Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. J Gen Virol 74:1093–1102.

Stuyver L, Claeys H, Wyseur A, Arnhem WV, De Beenhouwer H, Uytendaele S, Beckers J, Matthijs D, Leroux-Roels G, Maertens G, De Paepe M. 1996. Hepatitis C virus in hemodialysis unit: molecular evidence for nosocomial transmission. Kidney Int 49:889–895.

Thompson JD, Higgins DG, Gibson TJ. 1996. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nuc Acids Res 22:4673–4680.

Young KK, Resnick RM, Myers TW. 1993. Detection of hepatitis C virus RNA by a combined reverse transcription-polymerase chain reaction assay. J Clin Microbiol 31:882–886.